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Dioscin suppresses human laryngeal cancer cells growth via induction of cell-cycle arrest and MAPK-mediated mitochondrial-derived apoptosis and inhibition of tumor invasion

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ABSTRACT

The anti-cancer effects of dioscin have been widely reported. However, its effect on laryngeal cancer remains unknown. In the present paper, our results showed that dioscin markedly caused cell apoptosis and DNA damage, increased reactive oxygen species (ROS) level, induced S-phase arrest, and inhibited invasion of human laryngeal cancer HEp-2 and TU212 cells. Mechanism investigation showed that dioscin markedly up-regulated p53 level, and down-regulated cyclin-dependent kinase 2 (CDK2) and Cyclin A levels. In addition, dioscin significantly down-regulated the levels of p-ERK, Bcl-2, up-regulated the levels of p-JNK, p-p38, Bax, cleaved caspase-3/-9, and caused Cytochrome c release. Furthermore, U0126, an ERK1/2 inhibitor, markedly down-regulated Bcl-2 level, up-regulated the levels of Bax, cleaved caspase-3/9, and enhanced Cytochrome c release inducted by dioscin. While, SP600125 (one JNK inhibitor) and SB203580 (one p38 inhibitor) markedly up-regulated Bcl-2 level, down-regulated the levels of Bax, cleaved caspase-3/9, and obviously boosted Cytochrome c release induced by dioscin. Interestingly, dioscin also markedly down-regulated the levels of MMP2 and MMP9 associated with tumor invasion. Taken together, our study indicated that dioscin suppressed laryngeal cancer cells growth via inducting cell-cycle arrest, MAPK-mediated mitochondrial- derived apoptosis and inhibiting tumor invasion, which could be used as one potential candidate for the treatment of laryngeal cancer in the future.

1. Introduction

Head and neck cancer, the sixth commonest type of cancer worldwide, accounts for approximately a global incidence of 700,000 cases per year (Nix et al., 2005). Laryngeal squamous cell carcinoma (LSCC) is one of commonest malignancies which accounts for approximately 25% of the malignant head and neck tumors (Mirisola et al., 2011). In the United States, laryngeal cancer is estimated for almost 0.85% of all new cases of malignancies, and causes 0.65% of all cancer deaths in 2008 (Jemal et al., 2008). The 5-year survival rate is in the range of 33–57% (Andry et al., 2005), which has not been improved substantially over the past 25 years (Liao et al., 2009). Currently, Chemotherapy has been widely used for the treatment of laryngeal cancer (Urba et al., 2006). However, some anti-tumor drugs including imatinib, sunitinib and sorafenib have some serious side effects (Force and Kerkela, 2008; Schmidinger et al., 2008). Thus, exploration of new drugs with high efficiency and low toxicity for the treatment of laryngeal cancer is urgent.

Traditional Chinese medicines (TCMs), with high efficiency and low toxicity, have been performing the major roles in protecting health and controlling various diseases in China for thousands of years (S.P.Wang et al., 2012; Z.Y. Wang et al., 2012; Xu and Yang, 2009; Xu et al., 2014). Some natural products including coronaridine, paclitaxel extracted from medicinal herbs have been widely used for the treatment of laryngeal cancers (Liu et al., 2008). Thus, exploration of effective natural products from medicinal plants to treat laryngeal cancer is reasonable.

Dioscin (Dio, shown in Fig. 1A), a natural saponin, is an active ingredient from some medicinal plants (Lu et al., 2012). Pharmacological studies have demonstrated that dioscin can regulate

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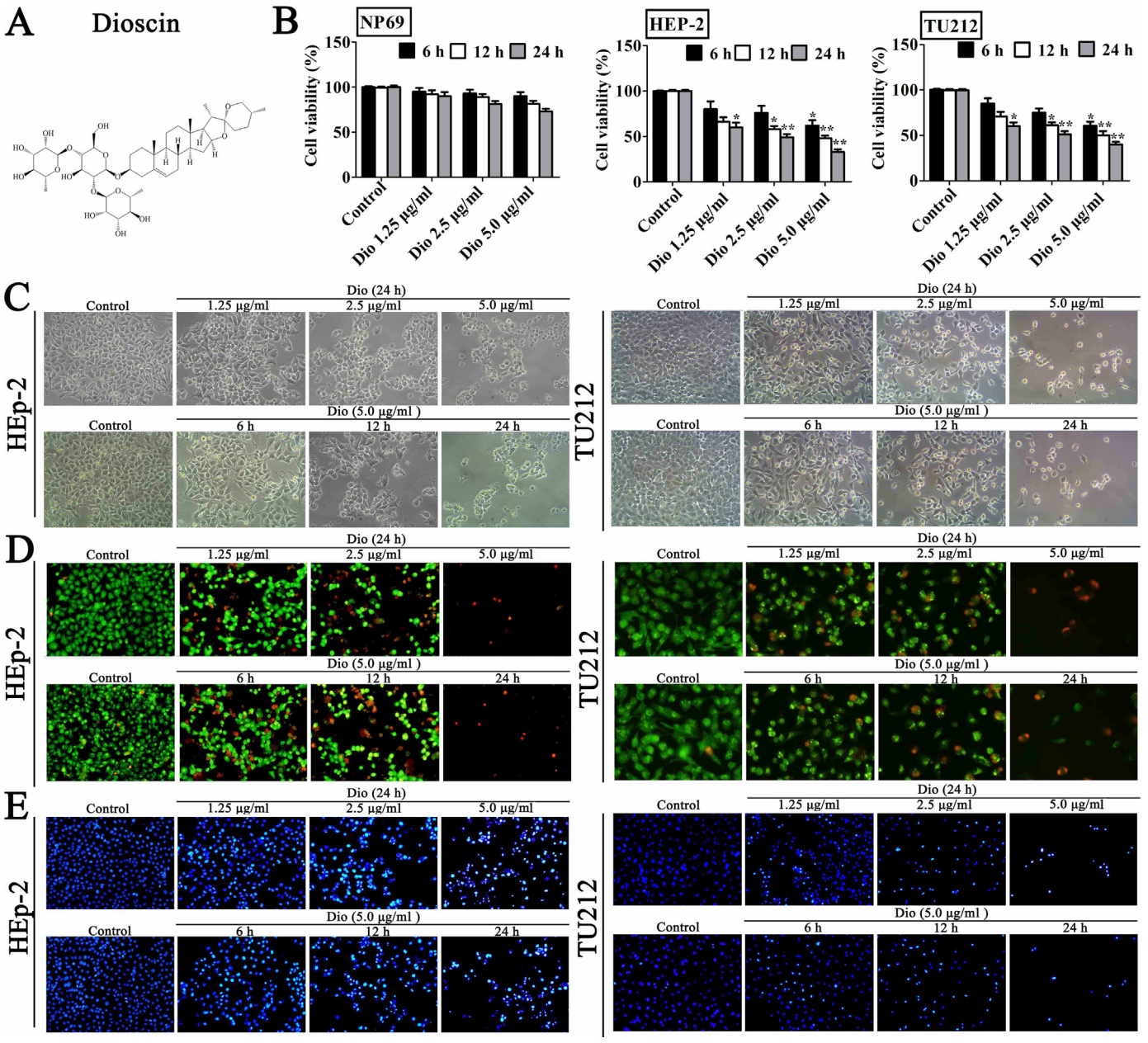

Fig. 1. (A) Chemical structure of dioscin. (B) Impacts of dioscin on the cell viabilities of NP69, HEp-2 and TU212 cells detected by MTT assay. (C) The morphological changes of the cells treated by different concentrations of dioscin (1.25, 2.5 and 5.0 μg/ml) for 24 h or with 5.0 μg/ml of dioscin for 6, 12 and 24 h (100 ×, final magnification). (D) Fluorescence images of HEp-2 and TU212 cells stained by AO/EB (100 ×, final magnification); (E) Fluorescence images of HEp-2 and TU212 cells stained by DAPI (100 ×, final magnification). Data are presented as mean ± S.D. ($n=5$). $^{**}P<0.01$ compared with control group.

hyperlipide (Li et al., 2010) and induce autophagy (Hsieh et al., 2013), and has anti-fungal (Cho et al., 2013), anti-obesity (Kwon et al., 2003), anti-virus (Aquino et al., 1991), anti-inflammatory (T.J. Wang et al., 2007; Y. Wang et al., 2007b), lipid-lowering (Sautour et al., 2004), and hepatoprotective (Zhao et al., 2012) activities. Furthermore, it has been widely used as one major raw material for the synthesis of steroid hormone drugs (Brautbar and Williams, 2002). In addition, dioscin also shows cytotoxic effects on several kinds of human cancer cell lines (Chen et al., 2014; Wei et al., 2013; Hsieh et al., 2012; S.P. Wang et al., 2012a; Z.Y. Wang et al., 2012b). However, to our best of knowledge no papers describing the anti-cancer effects of dioscin on laryngeal cancer have been published.

The aim of the present study was to investigate the effects and possible mechanism of dioscin against laryngeal cancer in vitro.

2. Material and methods

2.1. Chemicals and materials

Dioscin, with the purity > 98%, was prepared in our laboratory (Hu et al., 2013), which was dissolved with 0.1% dimethylsulfoxide (DMSO) for the experiments. Cell Cycle and Apoptosis Analysis Kits were all purchased from Beyotime Institute of Biotechnology (Shanghai, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Roche Diagnostics (Basel, Switzerland). Acridine orange (AO) and ethidium bromide (EB) fluorescent dyes, 4′,6′-Diamidino-2-phenylindole (DAPI) and DCFH-DA were purchased from Sigma Chemical Co.(St. Louis, MO, USA). Comet assay kit was purchased from Cell Biolabs, Inc. (San Diego, USA).